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## Antioxidant Activity of “Kolang Kaling”<sup>1</sup> Jam which is added with “Pucuk Merah” (*Syzygium oleana*) Fruit Juice

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### ABSTRACT

“Kolang kaling” is the endosperm of *Arenga pinnata* seed that contains galactomannan so it can be made into jam. This research was aimed to study the effect of “Pucuk merah” fruit juice as a natural colorant and antioxidant sources on antioxidant activity of the jam. This research used an experimental design with different levels of the juice of “Pucuk merah” fruit (6%, 8%, 10%, and 12%). Observations were anthocyanin level (pH differential method), total phenol (by Follin-Ciocalteu method) and antioxidant activity (by DPPH method) of the jam. It was found that the addition of the concentration level of “Pucuk merah” fruit juice had a statistically significant effect on the anthocyanin and total phenol level of the jam. The more the juice was added, the higher the anthocyanin level ( $2.82 \pm 0.13$  ppm;  $3.76 \pm 0.29$  ppm;  $3.88 \pm 0.11$  ppm; and  $4.20 \pm 0.52$  ppm, respectively) and the higher the total phenol level ( $382 \pm 1.53$  mg GAE/100g;  $431 \pm 3.06$  mg GAE/100g;  $540 \pm 1.53$  mg GAE/100g and  $547 \pm 6.43$  mg GAE/100g, respectively). By using sensory evaluation, the best product was the addition of 12% of the juice; the antioxidant activity was 628 ppm.

## 1. INTRODUCTION

### 1.1. Research Background

Jam is intermediate moisture food made from the processing of fruits, sugars or without the addition of permissible food additives [1]. The formation of gel during the processing of the fruit is highly dependent on the pectin content in the fruit pulp. In addition, the acidity and sugar added greatly determine the quality of the gel that is formed. Some fruits contain high pectin, so there is no need to add pectin to the fruit pulp in the gel formation process [2]. Pectin is a compound that includes hydrocolloids.

Hydrocolloid is a water soluble polymer, capable of forming colloids and capable of thickening a solution or forming a gel from the solution. Gradually the term hydrocolloid which is short for hydrophilic colloids is replacing the term gum because it is considered the term gum is too broad meaning. Gums are molecules with high molecular weight hydrophilic as well as hydrophobic, usually colloidal and in the appropriate developer material can form gel, solution or thick suspension at very low concentrations [3].

Hydrocolloid is widely used as a thickener or as a gelling agent so as to extend the shelf life of food. The thickening effect is mainly given by carboxymethyl cellulose, methyl cellulose and

hydroxypropylmethyl cellulose, guar gum, locust bean gum, tara gum, konjac maanan, gum tragacanth, gum ghatti and gum Arabic. Commonly used gelling agents include modified starch, agar, carrageenans, pectins, gellan gum, alginate and methyl and hydroxypropylmethyl celluloses [4].

As in Ref. [5], the nature of gel formation varies from one type of hydrocolloid to another depending on the type. Gel may contain 99.9% water but have more characteristic properties such as solids, particularly elasticity and rigidity. In principle, the formation of a hydrocolloid gel occurs due to the formation of a net or a three-dimensional network by a primary molecule that spans the entire volume of the gel formed by trapping a certain amount of water in it. Cross-linking of polymers consisting of long-chain molecules in sufficient quantities will then form a continuous three-dimensional building so that the solvent molecules will be trapped, immobilizing solvent molecules and forming rigid and firm structures that are resistant to particular forces or pressures. Gelation is a phenomenon involving incorporation, or the occurrence of crosslinking between polymer chains.

“Kolang-kaling” is an endosperm of seed of *Arenga pinnata* that is soft and chewy in clear white. [6] “Kolang-kaling” contains gum consisting of galactomannan. Galactomannan is a polysaccharide composed of galactose and mannose and has a soluble water property. Galactomannan is a compound that

includes hydrocolloids [7]. So “kolang-kaling” could potentially be the basic ingredient of jam, so galactomannan can replace the function of pectin.

However, the color of the jam has not well, so it was necessary to add fruit that has color to make the jam more interesting.

### 1.2. Literature Review

Galactomannan is a carbohydrate reserve that also serves to regulate the amount of water in seeds during germination [8]. In addition to as storage of carbohydrates, galactomannan also serves to store water to prevent the occurrence of drought in plants [9]. galactomannan is a heterogeneous polysaccharide consisting of a main chain of beta (1,4) -D manopiranos with 1 unit of alpha-D-galactopiranos branch attached to the alpha-position (1,6)[10]. the basic structures that build galactomannan are galactose and mannose [9].

The “Pucuk merah” is usually used as a hedge plant, as a protective crop on the side of the road or as an ornamental plant. This plant is one family with the guava plant that belongs to the family Myrtaceae. The fruit is small and dark red like blackberries. Anthocyanins in “Pucuk merah” are suspected was cyanidine-glycoside compounds. The content of anthocyanin in “Pucuk merah” was 462.51 mg L<sup>-1</sup> [11]. The anthocyanin content in the fruit has the potential as a natural antioxidant and natural colorant that has beneficial to health. Anthocyanins belong to a pigment called flavonoids that are generally soluble in water. The word anthocyanin comes from the Greek word “anthos” meaning flowers and “kyanos” which means dark blue [12]. Anthocyanin is a natural pigment with red, purple and blue color variations that are abundant in nature and safe to consume. It dissolves in water, and easy to use in food [13].

### 1.3. Research Objective

This research aimed to study the effect of “Pucuk merah” fruit juice addition on the antioxidant activity of “Kolang kaling” jam.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The main ingredient used in this research was “Kolang kaling” obtained from traditional market and “pucuk merah” as natural colorant obtained from region around Andalas university campus.

### 2.2. Experimental Design of this Research

Experimental design used in this research with 4 level of juice of “Pucuk merah” fruit (6%, 8%, 10% and 12%). The Data were analysed by using analysis of Variance (ANOVA). If there was a statistically significant difference, then proceed with Duncan’s New Multiple Range Test (DNMRT) at a 5% significance level. [14][15]

### 2.3. Production of “Pucuk Merah” Fruit Juice

“Pucuk merah” fruit are sorted and washed with water, then added with water (3:1) and crushed using a blender then filtered.

### 2.4. Production of “Kolang Kaling” Pulp

Refers to the method of making “kolang kaling” pulp as in Ref. [16].

### 2.5. Methods of Analysis

#### 2.5.1. Determination of pH: Using pH meter

#### 2.5.2. Determination of Anthocyanin : using method of pH differential [17], modified

Mixed sample, ethanol and HCL (1 ml: 9 ml: 1 ml) and then the mixture was homogenized. After that were mixed with solution of potassium chloride buffer (pH 1; 0.4 mL) and solution of sodium acetate buffer (pH 4.5; 4 mL), and then the mixture was measured its absorbance at  $\lambda$ 510 nm and  $\lambda$ 700 nm used a UV-Vis spectrophotometer. The absorbance was determined by calculation as  $A = [(A_{510} - A_{700}) \text{ pH } 1.0] - [(A_{510} - A_{700}) \text{ pH } 4.5]$  coefficient of anthocyanin was 26,900. Total anthocyanin was counted as cyanidin-3-glucoside with the following equation.

$$A = \text{absorbance}$$

$$MW = \text{Cyanidin-3-glucoside molecular weight (449.2 Da)}$$

$$DF = \text{Dilution Factor}$$

$$V = \text{volume (mL)}$$

$$103 = \text{conversion factor (from g to Mg)}$$

$$E = \text{absorbance molar Cyanidin-3- glucoside (26,900)}$$

$$L = \text{length of path (1 cm)}$$

$$M = \text{weight of sample (g)}$$

#### 2.5.3. Determination of Total Phenol: [18]

Dissolving 10  $\mu$ L of sample and 790  $\mu$ L of distilled water in the reaction tube, then were added with 50  $\mu$ L of Follin-Ciocalteu reagents and vortexed. After 1 minute, added with 150  $\mu$ L of sodium carbonate 20% solution then vortex and place at dark room for 120 minutes. Absorbance was determined at 750 nm and the concentration of the total polyphenol was counted from the curve calibration. Results are expressed as mg/L Gallic Acid Equivalents (GAE).

#### 2.5.4. Sensory analysis. [19]

Sensory analysis is a test that uses the human senses as a tool. This test is often used to determine agricultural and food commodities quality. The sensory analysis test used in this study is a hedonic test (preference test) by panellists. The test conducted is the acceptance test that each panellist is required to express a response about the product presented. The scale of preference was 1-5, that explanation was (1) very not like, (2) not like, (3) average, (4) like and (5) very like.

#### 2.5.5. Determination of Antioxidant Activity: DPPH Method (IC50) [20]

One g of sample dissolved with 10 ml of methanol and homogenized, it was taken the solution (0,5; 1,0; 1,5; 2,0 ml), diluted with methanol and distilled water (1:1) until the volume of 10 ml. Took 1 ml of each and added 1 ml of DPPH, leave on for 15 minutes. The solutions were measured by a spectrophotometer with a wavelength of 517 nm. The inhibition percentage of each was counted by the equation.

$$\text{Note: } A = \text{Absorbance of blank}$$

$$B = \text{Absorbance of sample}$$

### 3. RESULT AND DISCUSSION

Table 1 show that juice of “Pucuk merah” fruit contain anthocyanin as much as  $21.32 \pm 1.51$  ppm, the total phenol as much as  $832 \pm 2.08$  mg GAE g<sup>-1</sup> and the pH was  $4.58 \pm 0.02$ . The pH of “Kolang kaling” was  $3.45 \pm 0.008$ .

Anthocyanins are a group of plant pigments that soluble in water that were commonly found in different types of fruits and vegetables. The pigment has a color ranging from pink, red, purple and blue that has high potential to be used as a natural colorant, replace synthetic colorant. However, anthocyanins have unstable properties on processing and storage. Several factors that influence the stability of anthocyanins are environment, pH, sugar, temperature, enzymes, light and metal ions [21].

Table 1 .Analysis of Juice of “Pucuk Merah” Fruit And “Kolang Kaling” Puree

No	Variable	“Kolang Kaling” Puree	“Pucuk Merah” Fruit Juice
1	Anthocyanin (ppm)	-	$21.32 \pm 1.51$
2	Total Phenol (mg GAE/100 g)	-	$832 \pm 2.08$
3	pH	$3.45 \pm 0.08$	$4.58 \pm 0.02$

There are 18 types of anthocyanins that have been found but only six that play an important role in food and are often found, namely pelargonidin (orange and salmon), cyanidine (magenta and crimson), delphinidine (purple, mauve and blue, peonidine (magenta), petunidine (purple) and malvidine (purple) [22]. Ref.n [23] reported that Cyanidine is found in the fruit of “Senduduk” (*Melastome malabathricum*) and Malvidin is found in the rind of the “Jamblang” (*Syzygium cumini*) fruit.

Polyphenols are commonly found in fruit. Polyphenol per 100 g of fresh weight of pears, apples, grapes, berries and cherries contain up to 200-300 mg, processed products from this fruit, and still contains polyphenols in large quantities.[24]. As in [25] reported that the total phenolic content in freshly processed jams of strawberries with different maturity levels is almost the same and decreased during storage. The total phenolic of strawberry fruit, freshly processed jam, jam stored for 3 months at 4 0C and jam stored for 3 months at 20 0C were 184 mg/100g, 95 mg/100g, 97 mg/100g and 89 mg/100g respectively. Anthocyanin are a group of flavonoid is also included in the phenol group that has potential as antioxidant.

From the raw material test found that the pH of “Kolang kaling” puree was  $3.45 \pm 0.08$  whereas “Pucuk merah” fruit juice was  $4.58 \pm 0.02$ . This shows that the two main ingredients to be used are not sufficiently acidic, so in the production of jams must added with citric acid. The composition of water-soluble polysaccharide such as galactomannan, sugar and acids are essential in the formation of the gel.

#### 3.1. The value of pH of Jam

The pH value of this jam ranges from 3.4 to 3.5. (Table 2). Based on the analysis of variance showed that the addition of fruit juice gave no significant effect on the pH value of jam. This is due to the addition of citric acid was in the same amount of 0.2%. Measurement of pH is one of the variables associated with durability of a product. The desired optimum pH value in the

manufacture of jam ranges from 3.2 to 3.5 [2]. Table 2 shows that the pH value of this jam meets the requirements of the formation of the gel in all treatments. The pH value is often used as an indicator of food damage because the pH value is associated with the growth of spoilage microbes. Microbes can grow on food at a neutral pH.

Table 2. The Value of pH of “Kolang Kaling” Jam

No	“Pucuk Merah” Fruit Juice Addition (%)	pH Value
1	6%	$3.4 \pm 0.07$
2	8%	$3.5 \pm 0.02$
3	10%	$3.5 \pm 0.04$
4	12%	$3.5 \pm 0.02$

#### 3.2. Anthocyanin of Jam

Temperature and pH effect on stability of color. Increasingly heating temperature can cause loss glycosyl of the anthocyanin by hydrolysis of the glycosidic bond. The resulting compound is less stable and causes the color loss on anthocyanin of Rosella extract.[26]. Anthocyanin had positively correlated with chroma and negative correlated with the value of Hue [25].

Reducing anthocyanin can damage the color of the jam. Because of the temperature effected toward anthocyanin changes, so the fruit juice mixed with puree of “kolang kaling” at 50 0C. Although the use of these temperatures still has an effect on changes in anthocyanin content, but the decline is relatively small.

The use of 50 °C could reduce anthocyanin content by 17% [26]. Ref. [11] showed that using temperature up to 700C, degradation of the color of anthocyanin less than 4%, and use 1000C of temperature cause degradation of the color of anthocyanin as much as 31%, of extract by using a mixture of water and citric acid of 3%.

Table 3 show that the anthocyanin content range between  $2.82 \pm 0.13$  to  $4.20 \pm 0.52$  ppm. Based on the variance analysis indicated that the juice addition has significantly affect ( $\alpha < 0.05$ ) on anthocyanin of the jam. The results of the analysis were shown in table 2. The analysis showed that the higher the addition of juice cause anthocyanin levels in the jam increased significantly. The lowest anthocyanin is present in jam with 6% addition of fruit juice, whereas the highest anthocyanin is in jam with the addition of 12% fruit juice. There was a statistically significant difference between the addition of 6% fruit juice with the addition of 8%, 10% and 12% fruit juice. There was no statistically significant difference between the addition of 8% fruit juice with 10% and 12% fruit juice on the anthocyanin content of jam

Table 3. The Total Anthocyanin of “Kolang Kaling” Jam

No	“Pucuk Merah” Fruit Juice Addition (%)	Value (ppm)
1	6%	$2.82 \pm 0.13$ a
2	8%	$3.76 \pm 0.29$ b
3	10%	$3.88 \pm 0.11$ b
4	12%	$4.20 \pm 0.52$ b

Means within the same column with different superscripted letter show a significant difference

The “Pucuk merah” contains anthocyanin of 21.32 ppm, which affects the anthocyanin content of the jam. There are several benefits of using food containing anthocyanin, such as natural dyes that can replace synthetic dyes, harmless to the body and function as an antioxidant. As in [11] reported that anthocyanin content in “Pucuk merah” was 440 to 447 mg L<sup>-1</sup>, it was higher than this researched. Presumably this is related to the concentration of the extract, used crushing fruit of “Pucuk merah” made from mixing water and fruit (3: 1).

There are several advantages of using anthocyanin as a natural colorant in foods or beverages, that were safe and it has and also believed to play a role in biological systems, including the ability of free radical scavenging. [11].

### 3.3. Total Phenol of Jam

The result of variance analysis show that the juice addition had significantly affect at  $\alpha < 0.05$  on the phenol of “Kolang kaling” Jam. The results of the analysis can be seen in Table 4. The range of the total phenol was  $382 \pm 1.53$  to  $547 \pm 6.43$  mg GAE/100g. The more the juice addition, the higher the total of phenol in “Kolang-kaling” jam, it is influenced by the total phenol contained in the juice.

Table 4 show the total phenol in “Kolang kaling” with added of 6% “Pucuk merah” fruit juice was lowest and total phenol in the jam with added of 10% “Pucuk merah” fruit juice was highest. The addition of fruit juices 10% and 12%, did not show statistically significant differences. There was a statistically difference of total phenol between the jam with the addition of 6% fruit juice and the addition of 8%, 10% and 12%, between the addition of 8% of the juice and the addition of 10% and 12% fruit juice.

Phenol is sometimes called phenolic, is a chemical compound containing OH groups which is more soluble in water. As in [27] the total phenolics in *Melastoma malabathricum* leaves extract using water solvents, chloroform and methanol were 3344 mg GAE/100g, 92 mg GAE/100g and 3055 mg GAE/100 g, respectively. This data show that total phenolic solubility is better by using a water solvent.

Based on the analysis of raw materials, fruit juice contains total phenol of 832 mg GAE /100 g, that was lower than total phenol in *Melastome malabathricum* leaves extract.

**Table 4.** The Total Phenol of “Kolang Kaling” Jam

No	“Pucuk Merah” Fruit Juice Addion (%)	Value (mg GAE/100 g)
1	6%	$382 \pm 1.53$ a
2	8%	$431 \pm 3.06$ b
3	10%	$540 \pm 1.53$ c
4	12%	$547 \pm 6.43$ c

Means within the same column with different superscripted letter show a significant difference

Phenol compounds include a variety of plant-derived compounds, which have the same characteristic of an aromatic ring containing one or two hydroxyl, in which the role of several classes of phenol compounds is known, for example, lignin as the building material of cell walls, anthocyanin as the pigment of flower. The antioxidant activity of phenol compounds is formed because the ability of phenol compounds to form ions can give one electron to free radicals [13]

### 3.4. Sensory Analysis of Jam

Based on the variance analysis indicated that the juice addition had significantly different effect at  $\alpha < 5\%$  on the color and the taste of “Kolang kaling” jam, but there was not significantly different on the texture of the jam (Table 5). The more juice added to the “Kolang kaling”, the higher the level of color acceptance of the panelist. The level of panelist acceptance of color ranges from 3.5 to 4.3. There was no statistically significant difference between the addition of 6% juice with 8%; 8% with 10%; and 10% with 12%. There was a statistically significant difference between the addition of juice of 6% and 10%; 6% with 12%; and 8% with 12% on the color of the jam. The color produced on the “kolang kaling” jam with “pucuk merah” fruit juice added was a purplish red with varying degrees of color. The more juice is added resulting the darker the color of jam



**Fig. 1** “Kolang kaling” Jam added with juice of “Pucuk merah” Fruit Juice

The level of panelist’s acceptance of taste ranges from 3.5 to 4.0. There was no statistically significant difference between the addition of 6% juice with 8%; and 8% with 10% and 12%. There was a statistically significant difference between the addition of juice of 6% to 10% and 12%. The highest level of acceptance of the panelists was the addition of 12% of the juice. Taste is an important parameter to panelist’s acceptance, where flavour is the main parameter chosen by the consumer. The rate of addition of “Pucuk merah” fruit juice effect the sweet taste of jam produced.

**Table 5.** Sensory Analyses of “Kolang Kaling” Jam

No	“Pucuk Merah” Fruit Juice Addion (%)	Color	Texture	Taste
1	6%	3.5a	3.7	3.5a
2	8%	3.9ab	3.7	3.8ab
3	10%	4.1 bc	3.8	3.9 b
4	12%	4.3 c	3.9	4.0 b

Means within the same column with different superscripted letter show a significant difference

In addition to color and taste, texture has an important role in sensory reception. The acceptance value of textures ranges from 3.6 to 3.9. Based on statistical analysis showed that the addition of “Pucuk merah” fruit juice did not differ significantly to the texture of jam. Although there are differences in numbers, but statistically shows no significant difference. Based on the sensory test it is concluded that the panelists prefer jam with the addition of the most fruit juice, which is 12%. So the antioxidant analysis

is only done on the best product by sensory product with the addition of fruit juice 12%.

### 3.5. Activity of Antioxidant of Jam

Activity of antioxidant is influenced by the content of phenol in the material. Anthocyanins are flavonoid group which is also part of the phenol compound. There was correlation between antioxidant activity (DPPH method) and total phenol content ( $R^2 = 0.745$ ), and antioxidant activity and flavonoid content ( $R^2 = 0.759$ ) on the extract of *Trifolium pretense* L [28]. A stronger relationship was reported by [29] the correlation between activity of antioxidant (IC50) and total phenol content in extract of *Mimusops elengi* was 0.9714, and the correlation between the activity of antioxidant and the total flavonoids content was 0.9993. The more the total phenol and anthocyanin contents in the material, the higher the antioxidant activity.

**Table 6.** The Activity of Antioxidant of “Kolang Kaling” Jam

No	“Pucuk Merah” Fruit Juice Addion (%)	IC <sub>50</sub> (ppm)
1	12%	628

All polysaccharides that are phosphorylated showed that a strong influence as an antioxidant and its strength is affected by the degree of substitution. The higher the degree of substitution, the stronger the antioxidant activity. Galactomannan is phosphorylated carbohydrate [30]. The raw material of this research was “kolang kaling”. As in [7] reported that “kolang kaling” that made from *Arenga pinnata* contains galactomannan. Increasing the maturity will increase the content of galactomannan content, that were 1.27%, 2.71% and 3.72%. Galactomannan is composed of mannose and galactose with a ratio of 2: 1; 3: 1 and 5: 1 (M/G), where the increasing of mannose proportion with increasing the maturity.

This study has analyzed the jam that has highest total phenol and anthocyanin content. Antioxidant activity was aqueous by determining IC50 values that are defined as the concentration of antioxidant compounds that cause the loss of 50% DPPH activity. Compounds having lower IC50 values have higher antioxidant activity. The analysis was performed on jam with the juice addition based in the best treatment (added 12%). Antioxidant activity of this jam (628 ppm) was lower than antioxidant activity of isolated pheophytin from green tea. As in [31] showed that IC50 concentration of isolated pheophytin from green tea was 573 mg/L.

“Kolang kaling” with the fruit juice addition with a concentration of 12% has an IC50 value of 628 ppm. IC50 values describe the concentration of test compounds that can capture radicals by 50%. Ref. [11] reported that IC50 of “Pucuk merah” fruit extract was 0.055% (550 ppm) using a mixture of water and citric acid as solvent. It is compared with antioxidant activity in “Kolang kaling” jam added with “Pucuk merah” fruit juice, showed a very small decrease, but it was only added 12% of the juice. Maybe it is related with antioxidant activity of galactomannan in “Kolang Kaling”. As in [32] has proven that water-soluble polysaccharides, which are rich in galactomannan, have antioxidant activity, with an IC50 of 330 µg mL-1.

According to Ref. [33] the smaller the IC50 indicate the higher the activity of antioxidant. A compound has a very active antioxidant if the IC50 value is less than 50 ppm, said to be active

if it is 50-100 ppm, is said to be less the antioxidant activity, if the IC50, 101-250 ppm, said weak if it has 250-500 ppm and said to be inactive if it is more than 500 ppm.

“Pucuk merah” contain high phenol compounds; have a large contribution to the role of antioxidants, while anthocyanins are part of the total phenol. Phenol is also called phenolic compounds which are bioactive compounds that are often found in agricultural products.

## 4. CONCLUSION

The addition of juice of “Pucuk merah” fruit has a statistically significant effect on the anthocyanin content and total phenol of the “Kolang kaling” jam added with “Pucuk merah” fruit juice, but there is no significantly difference on the value of pH. The more the juice was added the more anthocyanin and total phenol content. Based on sensory analyses of “Kolang kaling” jam, the addition of the juice of 12% is the best product with score value of color was 4.3, the texture was 3.7, and the taste was 3.5. The addition of “Pucuk merah” fruit juice, resulting the red color of “Kolang kaling” jam, where more juice is added, the red color of the jam gets darker. “Kolang kaling” jam with the addition of the juice of 12% (based on the best product) has an IC50 value of 628 ppm. Although IC50 above 500 ppm, it showed that the jam still has antioxidant activity. In addition to “Pucuk merah”, “kolang kaling” also contribute as a compound of antioxidant. Antioxidants are compounds that can delay, slow down and prevent oxidation processes, and can be used to protect food components that contain unsaturated fatty acids or have double bonds. Antioxidants can also be used to protect vitamins or pigment that also contains multiple bonds in the structure.

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